the metabolic vulnerabilities of *Rb*-deficient tumors but also suggests that cancer cells may be capable of evolving the means to counter these deficiencies.

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Development: Hippo Signalling Turns the Embryo Inside Out

Lineage decisions in development are thought to be primarily due to differential activation of transcription factors. However, cell position and subcellular organization of signalling also play a role. New studies of the Hippo pathway in the early mouse embryo show how.

Miguel Manzanares[1,*] and Tristan A. Rodriguez[2,*]

In the early mammalian embryo, the first cell-fate decision leads to the formation of the trophectoderm, which will form the placenta, and the inner cell mass, which will give rise to the embryo proper and yolk sac. It has long been proposed that the position in the embryo of the cells that will form the trophectoderm or the inner cell mass is key to this specification event. The reason behind this suggestion is that when the trophectoderm and the inner cell mass segregate, between the 8-cell and 16-cell stage, the embryo resembles a compacted ball of cells with some cells positioned on the outside surface of this ball and others embedded inside it. Those cells that lie on the outside will form the trophectoderm and are polarised with an apical domain enriched in proteins such as the atypical protein kinase C (aPKC) and the polarity protein Par3. By contrast, the cells that lie inside are apolar and will go on to form the inner cell mass (Figure 1) [1,2]. But how is this difference translated into the activation of trophectoderm and inner cell mass specific gene expression? Two new studies in this issue of *Current Biology* by the groups of Hiroshi Sasaki [3] and Janet Rossant [4] shed important new light on this question.

The first clue for an involvement of the Hippo pathway in mammalian pre-implantation development came from analysis of mice lacking the transcription factor *Tead4* [5,6]. Tead is a member of the Hippo pathway, a signalling system that is evolutionarily conserved from *Drosophila melanogaster* to mammals and controls organ size through cell proliferation [7,8]. When the pathway is activated, the Tead co-factors Yap and Taz (homologues of *Drosophila* Yorkie) are phosphorylated and excluded from the nucleus, therefore preventing transcription of target genes. In the blastocyst, the Tead4 protein is present in all nuclei; however, Yap is only localised to the nucleus of outside trophectoderm cells. Consequently, *Tead4* mutants specify an inner cell mass but do not form a trophectoderm and lack proper expression of key regulators of the trophectoderm lineage, such as *Cdx2*. Therefore, activation of the Hippo pathway represses the trophectoderm fate. The protein kinases Lats1/2 (homologues of *Drosophila* Warts), which phosphorylate Yap/Taz, are crucial for this process, but again show no differential expression between inner and outer cells [9].

These studies provided evidence for an involvement of the Hippo pathway in repressing trophectoderm fate in inside cells [9], but several important questions remained unanswered: first of all, it had not been established if Hippo signalling plays any part in the specification of the inner cell mass. An unequivocal answer to this question is provided by the Rossant and Sasaki groups, who analysed the effects of loss of function of two different Hippo pathway components that had not previously been studied during pre-implantation development, Nf2 (the homologue of *Drosophila* Merlin)

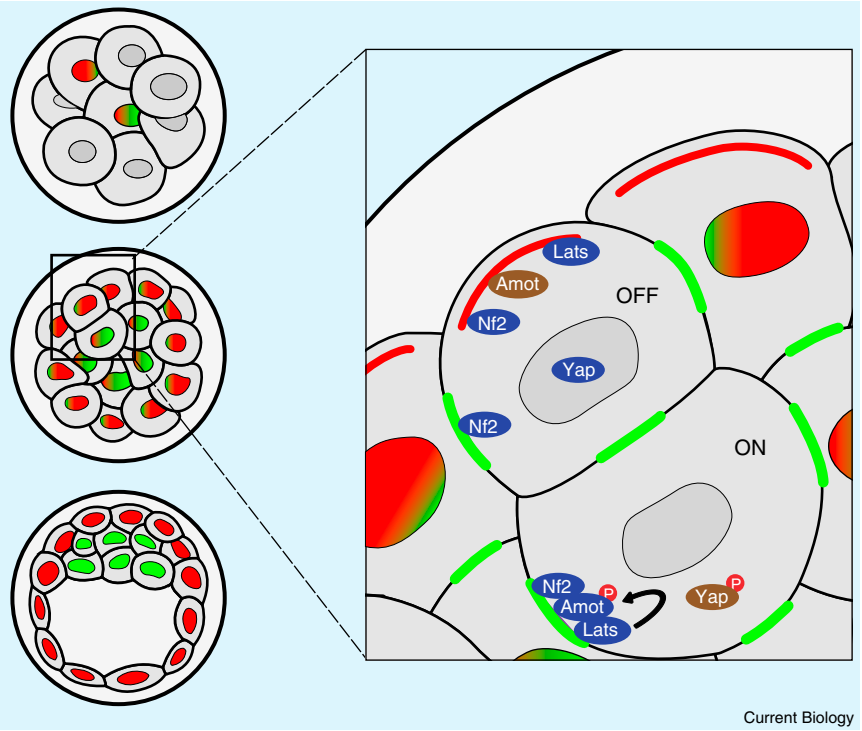

Figure 1. Inner-outer patterning of the early mouse embryo by subcellular shuffling of Hippo pathway components.

Starting at the 8-cell stage (top left), cells in the blastocyst progressively acquire inner (red) or outer (green) character through the compacted morula (middle left) and up to the blastocyst stages (bottom left) where the trophectoderm (TE) and inner cell mass (ICM) populations are clearly distinct. In inner cells of the morula (right hand panel), Amot and Lats associate with membranes though adherens junctions (green thickenings) where Nf2 is also present. In this context, Lats can phosphorylate Amot, and subsequently this complex phosphorylates Yap (in brown), excluding it from the nucleus. As a result, the Hippo pathway is activated ('ON'), resulting in an inner cell mass fate. Polarization of outer cells results in the trapping of Amot and Lats proteins to the apical complex (red thickening) what inhibits their association and the phosphorylation of Amot (in brown) by Lats. This configuration of the Hippo components turns the pathway off ('OFF') and precludes the phosphorylation of Yap, which can then move to the nucleus to activate the trophectoderm-specific transcriptional programme.

and Angiomotin (Amot). Strikingly, both the maternal/zygotic mutation of Nf2 and the combination of mutation of Amot and inhibition of its paralogue, Amotl2, lead to a loss of Hippo signalling that causes trophectoderm markers to be expressed in inner cell and, more importantly, the loss of expression of inner cell mass markers in these cells. Furthermore, in late blastocysts from Nf2 maternal/zygotic mutants the inner cells also begin to take on an epithelial character and express *Cdx2*, indicating that they are truly becoming trophectodermal. It is worth noting that these inner cells still lack the positional cues to establish proper apico-basal polarity, indicating that inhibition of Hippo signalling is likely to be downstream of cell polarity during trophectoderm formation.

But why does Hippo signalling become activated specifically in inner cells if none of the components of this pathway that have been studied so far are restricted to outer or inner cells? The answer we now discover, owing to the new work by Hirate *et al.* [3], is that Hippo is the read-out of the elusive 'polarity signal'. In a very elegant series of experiments, they provide evidence for a direct link between the cell polarity Par3–Par6–aPKC pathway and repression of Hippo activity. By disrupting Par6 or aPKC activity they show that the establishment of polarity is required to repress Hippo signalling in outer cells. Furthermore, using cell dissociation experiments, they demonstrate that cell adhesion allows for Hippo activation to take place in inside cells. This provides an idea of the cues that allow for differential Hippo activity, but the big breakthrough of this study is to show that Amot is the molecular link to these cues. They find that Amot is localised to the apical domain of outer cells and bound to actin where it is inactive, as it is sequestered by components of the polarity pathway. In contrast to this, in inner cells, Amot is found throughout the membrane, co-localised with adherens junctions from where it can mediate Hippo signalling (Figure 1).

So, if Amot is the link between the polarity and Hippo pathways, how is this link effected at the molecular level? To understand this, the Sasaki lab [3] carried out a deletion analysis of Amot and identify the domains that mediate interaction with adherens junctions, actin and other Hippo pathway components. They show that the Amot amino terminus mediates binding to components of the adherens junctions and to Lats2, while its coiled-coiled domain interacts with Nf2. This interaction with Lats2 is key to Amot's function. Lats2 phosphorylates Amot at Serine 176 and this step is required for Amot activity and only occurs in adherens junctions in inside cells. This phosphorylation suppresses actin binding, stabilises the interaction with Lats2, Nf2 and E-cadherin, and thus allows Amot to preferentially localise to adherens junctions rather than to the apical plasma membrane domain. The question that arises from these findings is why does Lats2 only phosphorylate Amot in inside cells? A possible reason can be found in the experiments by Cockburn *et al.* [4]. They find that Lats2 is apically localized in outside cells but evenly distributed throughout the cytoplasm of inside cells, suggesting that this protein is also sequestered, like Amot, by components of the polarity pathway.

Interestingly, the role of the Hippo pathway in the early mammalian embryo does not involve size control or proliferation — the classical functions ascribed to Hippo signalling. None of the loss of function mutations of components of the pathway studied so far (Tead4, Nf2, Amot/Amotl2, Lats1/2) results in changes in cell number, arguing that during pre-implantation development Hippo signalling has a novel role. Furthermore, Amot proteins have not been found in *Drosophila* [10], and thus their interaction with Hippo components would seem to be an innovation in vertebrates. The key novelty, however, found by these

studies [3,4] is that in the mammalian blastocyst, the subcellular redistribution of pathway components results in alternative cell fates.

What do these results tell us about the current models for trophectoderm and inner cell mass lineage segregation? Historically, two models have been put forward: the 'inside-outside model' suggests a cell's position leads to different amounts of cell contact and different microenvironments that are interpreted to establish cell fate [11], while the 'polarity model' suggests that the acquisition of cell polarity at the eight-cell stage is critical for lineage segregation [12]. The studies by the Sasaki and Rossant labs [3,4] argue that Hippo signalling is a sensor of both these processes, as it is inhibited by polarity in outside cells and activated by cell adhesion in inside cells.

So, is everything now solved regarding trophectoderm and inner cell mass specification? In our minds, a key unanswered question is if Hippo signalling is directly controlling the expression of lineage determinants or if its main role is to interpret the positional cues that the cell provides and translate these cues for the signals that specify fate. Yap is required to maintain pluripotency in embryonic stem cells [13], while in the inner cell mass Yap is excluded from the nucleus. Therefore, both 'On' and 'Off' states of Hippo signalling are equally compatible with the pluripotent programme. Also the main trophectoderm and inner cell mass lineage determinants (Cdx2 and Oct4) are initially co-expressed and only segregate to the trophectoderm and inner cell mass by the blastocyst stage [14], suggesting that additional cues to Hippo signalling are required to restrict these genes to their specific lineages. Understanding whether the state of the Hippo pathway is the only input that regulates the expression of these trophectoderm and inner cell mass determinants will start to provide an answer to this fascinating question.

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Cytokinins: Determinants of Sink Storage Ability

A new study demonstrates that storage organ formation can be induced in the axillary meristem of non-tuberizing plants by ectopic expression of the cytokinin biosynthetic gene LONELY GUY (LOG1).

J.A. Abelenda and Salomé Prat

During higher plant evolution, vegetative organs like leaves, stems or roots have acquired the ability to propagate asexually, for example, as observed in bulbs, corms, tubers and rhizomes, and this ability provides survival strategies during drought and freezing conditions that compromise plant's viability. These underground storage organs persist dormant in the soil during adverse periods, to sprout in the next favorable season and generate a new plant. Metabolic storage products accumulated in these organs, mostly in the form of soluble sugars or of starch, supply carbon and energy required for initial growth of the new shoot, hence making these organs an excellent caloric complement to human dietary needs. It is now widely accepted that early hominids fed on these organs during fallback episodes, and that the domestication of tuber-bearing species most likely preceded that of cereals and legumes. Reiterative selection for organs of large size gave rise to the modern potato and cassava cultivars, potato being nowadays the third crop in worldwide economic importance after wheat and rice, while cassava is one of the main staple crops in much of tropical Africa. Despite the enormous importance of these storage organs, little is known concerning how their formation is initiated or what restricts formation of these organs to a few plant species. The report by Eviatar-Ribak *et al.* published recently in *Current Biology* [1] shows that expression of the